AMENDMENTS TO THE SPECIFICATION

Pages 2-3, bridging paragraph, please replace with the following:

Type II diabetes, in contrast to type I, appears to arise at least in part from the inability of cells to respond to insulin. Insulin is responsible for stimulating glucose uptake into its target cells by a process which involves the translocation of the glucose transporter (GLUT) 4 isoform of glucose transporter from an intracellular vesicular compartment(s) to the plasma membrane. Thus, despite an ability to sense glucose and send proper signals (insulin) for glucose uptake, the afflicted individuals nonetheless suffer from poor glucose clearance and storage. The pathways that are responsible for insulin response unfortunately remain obscure.

Pages 4-5, bridging paragraph, please replace with the following:

In still a further embodiment, there is provided a method of identifying a subject at risk of developing diabetes comprising assessing the structure, function or expression of Fab1, Vac14 and/or Fig4 in cells of the subject. Assessing may comprise assessing expression, such as Northern blotting, quantitative RT-PCR, Western blotting or quantitative immunohistochemistry. Assessing may also comprise assessing activity, such as measuring $PI(3,5)P_2$, measuring $PI(3,5)P_2$ turnover, measuring $PI(3,5)P_2$ steady state levels, measuring $PI(3,5)P_2$ synthesis, measuring $PI(3)P$, or measuring protein kinase activity. Assessing may comprise assessing structure, such as (a) nucleic acid sequencing, including polymerase chain reaction (PCR)- and reverse transcriptase (RT)-PCR-based studies, (b) measuring antibody binding, such as radioimmunoassay (RIA), enzyme-linked immunosorbent assay (ELISA), Western blot or immunohistochemistry, and (c) high stringency nucleic acid hybridization. The method may further comprise obtaining a cell from the subject, such as a kidney cell, a liver cell, a leukocyte, an adipocyte, or a muscle cell. The method may further comprise subjecting the cell to stress prior to assessing expression or activity, such as osmotic stress. The method may

further comprise subjecting the cell to hormonal stimulation prior to assessing expression or activity, such as insulin stimulation.

Page 6, line 6, to page 7, line 27, please replace with the following:

FIG. 1 – Proteins with amino acid sequence identity to Vac14p exist in higher eukaryotes. Identical amino acids (black) and similar amino acids (gray) are highlighted. ~~(Left)~~ The left hand panel shows NH[[2]]$_2$-terminal sequence of *S. cerevisiae VAC14* and related ORFs were aligned using ClustalW (searchlauncher.bcm.tmc.edu:9331/multi-align/multi-align.html). Sequences were identified by searching the indicated databases via the BLAST algorithm (Altschul *et al.*, 1990). The *C. albicans* sequence was found in the *C. albicans* database (sequence-www.stanford.edu/group/candida/search.html). ORFs from *S. pombe* (EMBL/GenBank/DDBJ accession no. CAB08779.1), *A. thaliana* (EMBL/GenBank/DDBJ accession no. AAD12702.1), *C. elegans* (EMBL/GenBank/DDBJ accession no. CAB00043.1) and *D. melanogaster* (EMBL/GenBank/DDBJ accession no. AAF54829.1) were in the GenBank database (www.ncbi.nlm.nih.gov/BLAST). The *M. musculus* sequence was identified in the mouse EST database (www.ncbi.nlm.nih.gov/BLAST). The sequence shown is a consensus of two similar ESTs (EMBL/GenBank/DDBJ accession nos. BE573148 and BF162275). The *H. sapiens* sequence was in the human EST database (www.ncbi.nlm.nih.gov/BLAST). The consensus sequence of 14 similar ESTs from chromosome 16 (EMBL/GenBank/DDBJ accession nos. AL527155, AL535971, AL555680, AL556062, BE409891, BE696780, BE728471, BE893810, BE901196, BE937614, BF081182, BF091052, BF325708, and BG107035) is shown. The sequences contain at least 25% global identity and 42% global similarity *to S. cerevisiae VAC14*. ~~(Right)~~ The right hand panel shows COOH-terminal sequence of yeast Vac14p and similar ORFs were identified and aligned as in the left sequence. The *M. musculus* sequence is a consensus of 12 similar ESTs (EMBL/GenBank/DDBJ accession nos. AA036005, AA050423, AA058300, AA276168, AA497446, AA670618, BE862623, BF023070, BF237130, BF720417, BG079707, W09660). The *H. sapiens* sequence is hypothetical protein, FLJ10305 found on chromosome 16, deposited in the human

genome database (www.ncbi.nlm.nih.gov/genome/seq). One of the mouse ESTs (clone ID 468926) had been mapped to chromosome VIII (106 cM offset) with an inferred position on human chromosome 16 (16q22.1-qter).

FIG. 2 – Alignment of *Saccharomyces cerevisiae* (Sc) Fig4p and human (Hs) Fig4p. Amino acid sequences were aligned using the ClustalW program. The aligned sequences were divided into groups of ten and assigned a ~~color~~pattern bar based on their percent identity. The absence of a ~~colored~~pattern bar indicates a gap introduced by the ClustalW program to optimize the alignment.

FIG. 3 – The levels of ~~PI3P and PI3,5P2~~PI(3)P + PI(3,5)P_2 transiently change in response to hyperosmotic stress. Cells were labeled with myo-[2-[[3]]^{3}H]inositol for 12 hours and exposed to 0.9M NaCl for the indicated times. The dotted line indicates total ~~PI3P + PI3,5P2~~PI(3)P + PI(3,5)P_2.

FIG. 5 – The steady state levels of ~~PI3,5P2~~PI(3,5)P_2 are regulated ~~via~~by its rate of synthesis. Vps34p synthesizes ~~PI3P~~PI(3)P which is converted to PI3,5P2 by Fab1p. Vac7p and Vac14p are required for Fab1p activation under all conditions. Vac14p may have a specialized role in osmotic stress, while Fig4p is only required during osmotic stress. The mechanism of Vps34p activation is unkinown. The enzymes required for turnover may also be regulated; first inactivated, and subsequently stimulated.

FIG. 6 – Levels of ~~PI3P~~PI(3)P and ~~PI3,5P2~~PI(3,5)P_2 in the Class III mutant yeast strains after osmotic stress. Cells were labeled with myo-[2-3H]inositol for 12 hours and exposed to 0.9M NaCl for the indicated times. The lines shown in gray indicate PPI levels in a wild-type strain.

Pages 22-23, bridging paragraph, please replace with:

It will be desirable to purify polypeptides or variants thereof. Protein purification techniques are well known to those of skill in the art. These techniques involve, at one level, the crude fractionation of the cellular milieu to polypeptide and non-polypeptide fractions. Having separated the polypeptide from other proteins, the polypeptide of interest may be further purified using chromatographic and electrophoretic techniques to achieve partial or complete purification (or purification to homogeneity). Analytical

methods particularly suited to the preparation of a pure peptide are ion-exchange chromatography, exclusion chromatography; polyacrylamide gel electrophoresis; isoelectric focusing. A particularly efficient method of purifying peptides is fast protein liquid chromatography or even High Performance Liquid Chromatography (HPLC).

Page 23, lines 22-31, please replace with the following:

Various methods for quantifying the degree of purification of the protein or peptide will be known to those of skill in the art in light of the present disclosure. These include, for example, determining the specific activity of an active fraction, or assessing the amount of polypeptides within a fraction by sodium dodecyl sulfate/polyacrylamide gel electrophoresis (SDS/PAGE) analysis. A preferred method for assessing the purity of a fraction is to calculate the specific activity of the fraction, to compare it to the specific activity of the initial extract, and to thus calculate the degree of purity, herein assessed by a "-fold purification number." The actual units used to represent the amount of activity will, of course, be dependent upon the particular assay technique chosen to follow the purification and whether or not the expressed protein or peptide exhibits a detectable activity.

Page 24, lines 1-9, please replace with the following:

Various techniques suitable for use in protein purification will be well known to those of skill in the art. These include, for example, precipitation with ammonium sulphate, polyethylene glycol (PEG), antibodies and the like or by heat denaturation, followed by centrifugation; chromatography steps such as ion exchange, gel filtration, reverse phase, hydroxylapatite and affinity chromatography; isoelectric focusing; gel electrophoresis; and combinations of such and other techniques. As is generally known in the art, it is believed that the order of conducting the various purification steps may be changed, or that certain steps may be omitted, and still result in a suitable method for the preparation of a substantially purified protein or peptide.

Page 26, lines 22-28, please replace with the following:

The present invention also provides, in another embodiment, nucleic acids encoding Fab1p, Vac14p and Fig4p. Genes from human and yeast have been identified, and in some cases mouse as well. See, for example, ~~SEQ ID NOS~~SEQ ID NOs: 2, 4, 6, and 8 respectively. The present invention is not limited in scope to these genes, however, as one of ordinary skill in the could, using these nucleic acids, readily identify related homologs in these and various other species (*e.g.*, rat, rabbit, dog, monkey, gibbon, chimp, ape, baboon, cow, pig, horse, sheep, cat and other species).

Page 28, lines 1-9, please replace with the following:

As used in this application, the term "a nucleic acid encoding a Fab1p, Vac14p, Fig4p" refers to a nucleic acid molecule that has been isolated free of total cellular nucleic acid. In preferred embodiments, the invention concerns a nucleic acid sequence essentially as set forth in ~~SEQ ID NOS~~SEQ ID NO: 2, 4, 6, or 8 (mouse, human, zebrafish, or *C. elegans*, respectively). The term "as set forth in ~~SEQ ID NOS~~SEQ ID NO: 2, 4, 6, or 8" means that the nucleic acid sequence substantially corresponds to a portion of SEQ ID NO:2, 4, 6, or 8. The term "functionally equivalent codon" is used herein to refer to codons that encode the same amino acid, such as the six codons for arginine or serine (Table 1, below), and also refers to codons that encode biologically equivalent amino acids, as discussed in the following pages.

Page 53, lines 6-23, please replace with the following:

DNA clones for microinjection can be prepared by any means known in the art. For example, DNA clones for microinjection can be cleaved with enzymes appropriate for removing the bacterial plasmid sequences, and the DNA fragments electrophoresed on 1% agarose gels in TBE buffer, using standard techniques. The DNA bands are visualized by staining with ethidium bromide, and the band containing the expression sequences is excised. The excised band is then placed in dialysis bags containing 0.3 M

sodium acetate, pH 7.0. DNA is electroeluted into the dialysis bags, extracted with a 1:1 phenol:chloroform solution and precipitated by two volumes of ethanol. The DNA is redissolved in 1 ml of low salt buffer (0.2 M NaCl, 20 mM Tris, pH 7.4, and 1 mM ethylenediamine tetraacetic acid (EDTA)) and purified on an Elutip-D™ column. The column is first primed with 3 ml of high salt buffer (1 M NaCl, 20 mM Tris, pH 7.4, and 1 mM EDTA) followed by washing with 5 ml of low salt buffer. The DNA solutions are passed through the column three times to bind DNA to the column matrix. After one wash with 3 ml of low salt buffer, the DNA is eluted with 0.4 ml high salt buffer and precipitated by two volumes of ethanol. DNA concentrations are measured by absorption at 260 nm in a UV spectrophotometer. For microinjection, DNA concentrations are adjusted to 3 μg/ml in 5 mM Tris, pH 7.4 and 0.1 mM EDTA. Other methods for purification of DNA for microinjection are described in Palmiter *et al.* (1982); and in Sambrook *et al.* (1989).

Page 74, lines 12-21, please replace with the following:

Analysis of GFP-Fab1p ~~in vivo~~*in vivo*. Green fluorescent protein (GFP) was inserted into the FAB1 ORF in pRS426 by homologous recombination. To introduce FAB1 sequences at both the 5' and 3' ends, GFP was amplified by PCR from pGOGFP (Cowles ~~et al~~*et al.*, 1997) using the primers FABGFP-N (5'-GCT CAC ATG TCC GGT CGTCCT CCA CTG GTA CTT CAT CTG TGA TGG GTA AAG GAG AAG AAC TTT TC-3') and FABGFPC2 (5'-GCG ACG CAG TGC CGG TCA CGT GAC TTG TTG ATG TCG CTG TTG CGG ATC CCG GGC CCG CGG TAC CGT C-3'). The PCR product and AatII-linearized pRS426-FAB1 were cotransformed into yeast and resultant colonies were screened for GFP fluorescence. The pRS426-GFP-FAB1 plasmid was then transformed into various yeast strains and fluorescence was visualized with the same filter set used for quinacrine.